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Design, synthesis and evaluation of a novel cyclohexanamine class of neuropeptide Y Y1 receptor antagonists

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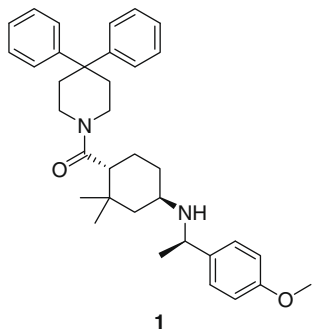
ABSTRACT

A novel series of cyclohexanamine derivatives was designed and synthesized as potent and selective human neuropeptide Y Y1 receptor antagonists. Modification of high-throughput screening hit compound **1** resulted in the identification of compound **3i**, which displays potent Y1 activity and good selectivity towards hERG K^+ channel and serotonin transporter.

Neuropeptide Y (NPY) is a 36-amino acid peptide identified in 1982 as a member of the pancreatic polypeptide family.[1] NPY is widely distributed in the central and peripheral nervous system, and has been implicated in the central regulation of feeding behavior and energy homeostasis.[2–5] Chronic central infusion of NPY into the brain results in body weight gain with hyperphagia, reduces energy expenditure, and increases lipogenic activity in the liver and adipose tissue.[5,6] In addition, NPY-deficient ob/ob mice are less obese and have reduced food intake compared with ob/ob mice.[7]

Five distinct NPY receptor subtypes (Y1, Y2, Y4, Y5, and mouse Y6) have been characterized.[8] Pharmacological data suggest that the Y1 receptor is a major feeding receptor.[8,9] In addition, we previously demonstrated that a series of aminopyridine Y1 antagonists inhibit NPY-induced food intake and spontaneous feeding after oral administration in rodents.[10,11] Therefore, antagonism of the Y1 receptor may have considerable therapeutic benefits in treating obesity. Several research groups interested in the Y1 receptor have reported various structural classes of Y1 antagonists.[12] Screening of Merck sample collections against human NPY Y1 receptor resulted in the identification of cyclohexanamine lead **1**, which has a K_i value of 7.4 nM. Subsequent optimization efforts focused on reducing human ether-a-go-go related gene (hERG) channel and human serotonin transporter (hSERT) inhibitory activities (Fig. 1). The synthesis and structure–activity relationships (SAR) of this novel cyclohexanamine class of Y1 antagonists are described.

The synthetic routes for the derivatives reported herein are described in Schemes 1–4. Intermediates **6**, **9a** and **9b** were prepared as shown in Scheme 1. The *tert*-butoxycarbonyl (Boc)-protected piperidone **4** was coupled with (2-methoxypyridin-5-yl)lithium to afford 4-arylpiperidine **5**, which was treated with benzene in the presence of $AlCl_3$ to give 4,4-diarylpiperidine **6**.[13] The secondary amine of **6** was alkylated with benzyl bromide followed by deprotection of the methyl group to furnish benzylamine **7**. Chlorination of the hydroxyl group of **7** using phosphorusoxychloride afforded 2-chloropyridine **8** in good yield. Removal of the benzyl



1

hY1 K_i = 7.4 nM
hERG IC_{50} = 432 nM
hSERT IC_{50} = 14 nM

Figure 1. Structure of compound **1**.

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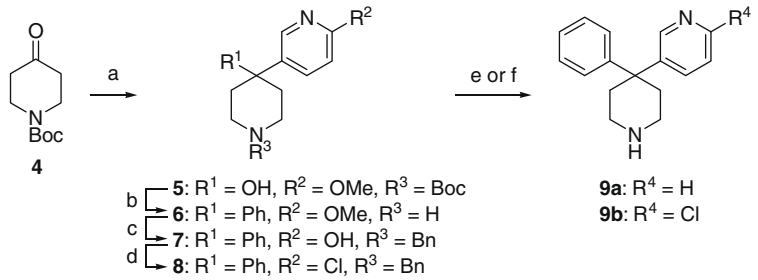
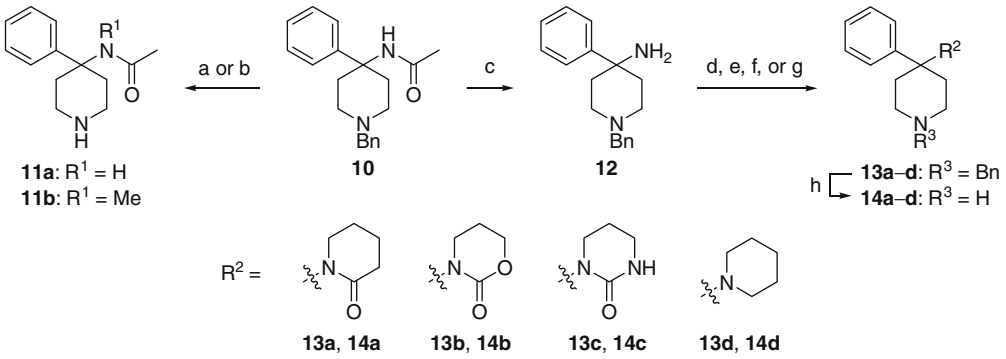
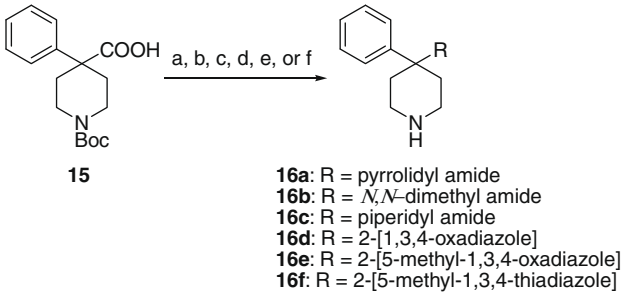


Scheme 1. Synthesis of intermediates **6**, **9a**, and **9b**. Reagents and conditions: (a) 5-bromo-2-methoxypyridine, *n*-BuLi, THF, −78 °C to rt, 12 h, quant.; (b) $AlCl_3$, benzene, CH_2Cl_2, 0 °C, 4 h, 72%; (c) (i) BnBr, TEA, MeCN, rt, 2 h, (ii) TMSCl, NaI, MeCN, reflux, 1 h, 90% over two steps; (d) $POCl_3$, reflux, 3 d, 83%; (e) H_2, 10 bar, 40 °C, H-cube, quant.; (f) ACECl, CH_2Cl_2, rt, 1 h, then MeOH, reflux, 1 h, 53%.



Scheme 2. Synthesis of intermediates **14a**–**d**. Reagents and conditions: (a) H_2, $Pd(OH)_2$, MeOH, rt, 2 h, quant.; (b) (i) MeI, NaH, DMF, rt, 1 h, (ii) H_2, $Pd(OH)_2$, MeOH, rt, 2 h, 74% over two steps; (c) 5 N HCl, reflux, 12 h, 68%; (d) (i) $ClCO(CH_2)_4Br$, TEA, $CHCl_3$, rt, 2 h, (ii) NaH, DMF, rt, 1 h, 88% over 2 steps; (e) (i) $ClCO_2(CH_2)_3Cl$, TEA, $CHCl_3$, rt, 1 h, (ii) NaH, DMF, rt, 1 h, 85% over two steps; (f) (i) $Br(CH_2)_3NHBoc$, K_2CO_3, DMF, 100 °C, 24 h, (ii) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 2 h, (iii) di(*N*-succinimidyl) carbonate, DIEA, $CHCl_3$, rt, 12 h, 39% over three steps; (g) glutaric dialdehyde, $Zn(BH_3CN)_2$, MeOH, rt, 12 h, 40%; (h) H_2, $Pd(OH)_2$, rt, 2 h, 90%.



Scheme 3. Synthesis of intermediates **16a**–**f**. Reagents and conditions: (a) (i) pyrrolidine, EDCI·HCl, HOBt, TEA, $CHCl_3$, rt, 12 h, (ii) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 2 h, 80%—quant. over two steps; (b) (i) 40% aq $NHMe_2$, EDCI·HCl, HOBt, TEA, $CHCl_3$, rt, 12 h; (ii) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 2 h, 96% over two steps; (c) (i) piperidine, EDCI·HCl, HOBt, TEA, $CHCl_3$, rt, 12 h; (ii) 4 N HCl, rt, 12 h, 70% over two steps; (d) (i) CDI, $CHCl_3$, 50 °C, 2 h, (ii) $NH_2NH_2{\cdot}H_2O$, rt, 12 h; (iii) DMFDMA, cat. TsOH, toluene, 100 °C, 12 h; (iv) TMSOTf, 2,6-lutidine, $CHCl_3$, rt, 12 h, 19% over four steps; (e) (i) CDI, $CHCl_3$, 50 °C, 2 h, (ii) $NH_2NH_2{\cdot}H_2O$, rt, 12 h; (iii) $(EtO)_3CMe$, 170 °C, microwave, 30 min; (iv) TMSOTf, 2,6-lutidine, $CHCl_3$, rt, 12 h, 49% over four steps; (f) (i) CDI, $CHCl_3$, 50 °C, 2 h; (ii) $AcNHNH_2$, 50 °C, 12 h; (iii) Lawesson's reagent, THF, reflux, 12 h; (iv) TMSOTf, 2,6-lutidine, $CHCl_3$, rt, 12 h, 61% over four steps.

and chloro groups of **8** was achieved by hydrogenation using H-cube[14] to give amine **9a**. Compound **8** was also converted to **9b** using 1-chloro-ethylchloroformate (ACECl).

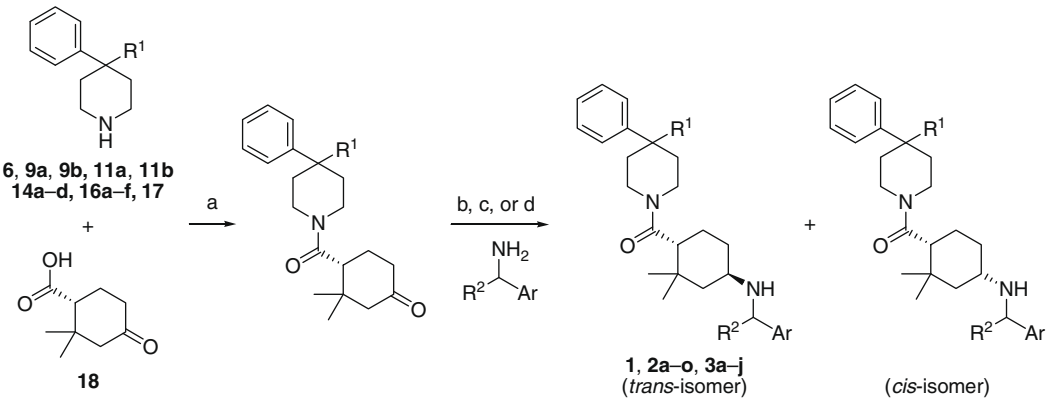
The synthesis of intermediates **11a**, **11b** and **14a**–**d** is illustrated in Scheme 2. The benzyl group of commercially available amide **10** was cleaved by hydrogenation to give amine **11a**. Methylation of **10** followed by removal of the benzyl group afforded **11b**. The amide group of **10** was hydrolyzed under acidic conditions to furnish amine **12**, which was converted to **13a**–**d** by conditions d, e, f or g as shown in Scheme 2. Deprotection of the benzyl group of **13a**–**d** gave amines **14a**–**d**.

The preparation of intermediates **16a**–**f** is outlined in Scheme 3. Commercially available carboxylic acid **15** was coupled with pyrrolidine followed by removal of the Boc group of the resultant amide to give amine **16a**. Amines **16b** and **16c** were synthesized in the same manner. Oxadiazole-containing piperidines **16d** and **16e** were prepared by coupling **15** with hydrazine followed by thermal condensation of the resultant hydrazide with dimethylformamide dimethylacetal (DMFDMA) or 1,1,1-triethoxyethane and subsequent deprotection of the Boc group. Coupling of **15** with $AcNHNH_2$ and subsequent treatment with Lawesson's reagent followed by cleavage of the Boc group yielded desired amine **16f**.

Synthesis of cyclohexanamine derivatives **1**, **2a**–**o** and **3a**–**j** is shown in Scheme 4. Compounds **6**, **9a**, **9b**, **11a**, **11b**, **14a**–**d**, **16a**–**f**, and commercially available 4,4-diphenylpiperidine (**17**) were condensed with acid **18**,[15] followed by reductive amination with the desired benzylamines to give the target compounds as a mixture of diastereomers. Finally, the desired *trans*-isomers **1**, **2a**–**o** and **3a**–**j** were obtained by preparative reversed-phase HPLC.[16,17]

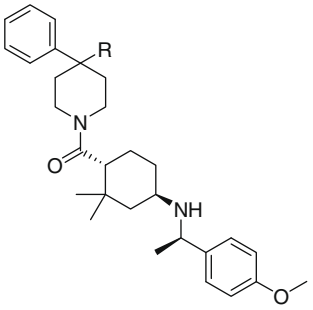
The cyclohexanamine compounds described herein were tested in a [^{125}I]PYY binding assay using LMtk$^-$ cell membranes expressing human recombinant Y1 receptors.[18] Selected compounds were evaluated for hERG K^+ channel inhibitory activity using the [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]-methanesulfonamide binding assay to assess QTc prolongation liability.[19] Inhibitory activity for hSERT was measured using the [^{3}H]imipramine binding assay.[20]

High-throughput screening of Merck sample collections against the human Y1 receptor resulted in the identification of **1**, which has a K_i value of 7.4 nM.[21] Compound **1** was found to have



Scheme 4. Synthesis of derivatives **1**, **2a–o**, and **3a–j**. Reagents and conditions: (a) EDCI·HCl, HOBt, TEA, $CHCl_3$, rt, 12 h; (b) $Zn(BH_3CN)_2$, MeOH, rt, 12 h; (c) $NaBH(OAc)_3$, AcOH, MeCN, rt, 12 h; (d) $NaBH(OOCH)_3$, HCO_2H, 1,2-dichloroethane, rt, 12 h, 5–40% over two steps.

Table 1
SAR of derivatives **1** and **2a–o**[a]



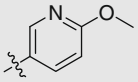
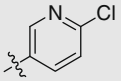
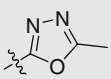
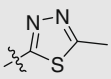
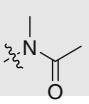
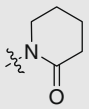
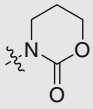
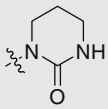
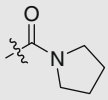
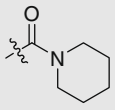
Compd	R	hY1[b] (K_i, nM)	hERG[c] (IC_{50}, nM)	hSERT[d] (IC_{50}, nM)
1		7.4	530	15
2a		29	9600	17
2b		24	520	14
2c		32	2100	8
2d		890	>10,000	—
2e		120	>10,000	—
2f		330	>10,000	—
2g		48	>10,000	26
2h		49	>10,000	14

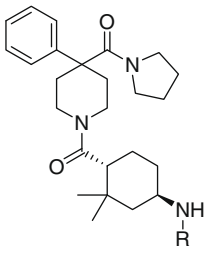
Table 1 (*continued*)

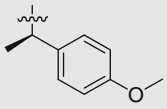
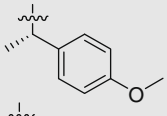
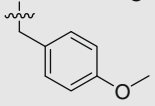
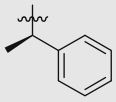
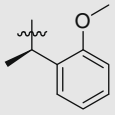
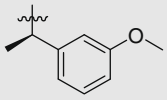
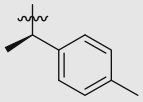
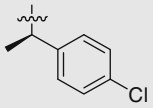
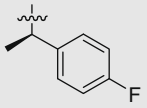
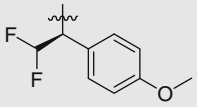
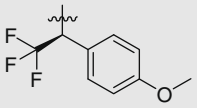
Compd	R	hY1[b] (K_i, nM)	hERG[c] (IC_{50}, nM)	hSERT[d] (IC_{50}, nM)
2i		18	>10,000	11
2j		42	>10,000	17
2k		45	>10,000	24
2l		3000	6700	—
2m		7.3	>10,000	17
2n		150	9000	—
2o		15	>10,000	38

[a] Values are the mean of two or more independent assays.
[b] Inhibition of [^{125}I]PYY binding to human recombinant Y1 receptor in CHO (NFAT-bla) cell membranes.
[c] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[d] Inhibition of [^{3}H]imipramine binding to human serotonin transporter in CHO cell membranes.

moderate hERG activity (IC_{50} = 530 nM) and potent hSERT inhibition (IC_{50} = 15 nM). Modification of the upper phenyl group of **1** was initiated to improve selectivity towards hERG and hSERT (Table 1). Pyridine derivatives **2a–c** were more than threefold less potent than the parent **1**. Replacement of the phenyl group with five-membered heterocycles was detrimental to Y1 potency, as shown by **2d–f**. Subsequently, the introduction of nonaromatic

Table 2
SAR of derivatives **3a–j**[a]



Compd	R	hY1[b] (K_i, nM)	hERG[c] (IC_{50}, nM)	hSERT[d] (IC_{50}, nM)
2m		7.3	>10,000	17
3a		1100	4700	–
3b		470	900	–
3c		160	>10,000	–
3d		790	>10,000	–
3e		210	>10,000	–
3f		10	>10,000	76
3g		27	1300	59
3h		44	>10,000	130
3i		22	>10,000	4800
3j		43	7400	>10,000

[a] Values are the mean of two or more independent assays.
[b] Inhibition of [^{125}I]PYY binding to human recombinant Y1 receptor in CHO (NFAT-bla) cell membranes.
[c] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[d] Inhibition of [^{3}H]imipramine binding to human serotonin transporter in CHO cell membranes.

functional groups in place of the phenyl ring was investigated. Acetamide compounds **2g** and **2h** were sevenfold less potent than compound **1**, and lactam **2i** displayed slightly decreased Y1 potency relative to **1**. Introduction of heteroatoms into the lactam or reduction of the carbonyl group as in **2j–l** led to further decreases in Y1 activity. Pyrrolidinyl amide analog **2m** was found to be equipotent to **1**, while replacement of the pyrrolidine with other secondary amines, as in **2n** and **2o**, resulted in reduced Y1 activity. The hERG and hSERT inhibitory activities were subsequently evaluated. All compounds except **2b** exhibited reduced hERG activity, probably due to the increased hydrophilicity of the derivatives. However, modification of the phenyl part did not affect hSERT inhibition. We therefore focused on modifying the benzyl amine part, using **2m** as a template.

The effects of the methyl substituent on the benzyl position of **2m** were examined (Table 2). (*S*)-Methyl substitution as in **3a** resulted in significant loss of potency, while removal of the methyl group was also detrimental to Y1 activity, as demonstrated by **3b**. Next, we investigated the effects of substituents on the phenyl ring. Removal of the methoxy group as in **3c**, and positional scanning with a methoxy group as in **2m**, **3d** and **3e**, revealed that 4-substitution is critical for Y1 potency. Variation of the 4-substituent on the phenyl ring was studied next. The methyl derivative **3f** had Y1 activity comparable to **2m** while maintaining negligible hERG activity. The chloro and fluoro derivatives **3g** and **3h** were four and sixfold less potent than **2m**, respectively. These results suggested that substitution of the 4-position by electron-donating groups increases Y1 potency. However, variation of 4-substituents had little impact on hSERT activity. Proposed pharmacophoric models for hSERT inhibition indicate that a positively charged amino group is critical for hSERT activity.[22] With this in mind, we next aimed to reduce the basicity of the amine, so the effects of fluorine substitution near the basic nitrogen on hSERT inhibitory activity were investigated. Indeed, the difluoromethyl derivative **3i** exhibited dramatically reduced hSERT activity while maintaining Y1 potency and decreased hERG activity. Further addition of a fluorine, as in **3j**, resulted in negligible hSERT activity, but also twofold less potent Y1 activity compared to **3i**. Considering the calculated pK_a values of **2m** ($pK_a = 10.25$), **3i** ($pK_a = 6.02$), and **3j** ($pK_a = 4.24$), reduction in pK_a values might be responsible for the observed reduction in hSERT activity.[23]

Having demonstrated good Y1 affinity and selectivity over hERG and hSERT, compound **3i** was tested in the [^{35}S]GTPγS binding assay.[24] In this functional assay, **3i** showed appreciable antagonistic activity ($IC_{50} = 280$ nM). In addition, **3i** was selective over other NPY receptor subtypes (Y2 IC_{50}: 1400 nM, Y4 IC_{50}: >10,000 nM, and Y5 IC_{50}: >10,000 nM).[25]

In summary, we have designed, synthesized, and evaluated a series of novel cyclohexanamine derivatives as NPY Y1 antagonists. The major focus of this study was to eliminate the hERG and hSERT activities of lead compound **1**. As a results, compound **3i** was found to have good Y1 potency and selectivity over hERG, hSERT and NPY receptor subtypes. Evaluation of the in vivo profile of **3i** and further optimization of this promising cyclohexanamine lead class are currently in progress.

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References and notes

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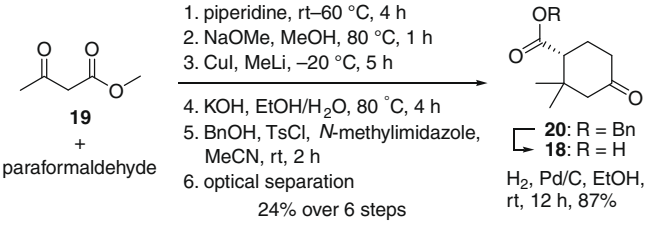
15. Compound **18** was synthesized as depicted below.



Compound **20** was resolved by CHIRALCEL AD-H (2 cm × 25 cm, 5 μm), eluting with 10% EtOH in hexanes. The first-eluted fraction was collected and hydrogenated to afford **18** as a single enantiomer. The absolute configuration of **18** was determined by comparing 3-{[2,2-dimethyl-4-oxocyclohexyl]-carbonyl}-1,3-oxazolidin-2-one derived from **18** with the authentic sample prepared according to procedures described in Usuda, H.; Kuramochi, A.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2004**, *6*, 4387.

16. The diastereomeric mixture of the target compounds was purified either by conditions a or b, described as follows. The desired *trans*-isomers were obtained as the first-eluted compounds in all cases: (a) Column: SunFire Prep C18 BD (3 cm × 5 cm, S-5 μm), eluent: a gradient of 0.1% aq HCOOH/0.1% HCOOH in CH_3CN = 90/10 to 10/90 over 10 min, flow rate: 40 mL/min; (b) XBridge Prep C18 OBD (3 cm × 5 cm, S-5 μm), eluent: a gradient of 0.05% aq NH_3/0.05% NH_3 in CH_3CN = 80/20 to 5/95 over 15 min, flow rate: 40 mL/min.
17. The relative configuration of compound **1** was determined by NOEs.
18. Binding of [^{125}I]PYY to membrane preparations was conducted using a minor modification of the method described in Ref.[11a]. In brief, the membranes were incubated in 0.2 ml of 20 mM HEPES buffer (pH 7.4) containing 0.1% bacitracin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% BSA and Hank's balanced salt solution (HBSS) in the presence of the test compound at various concentrations with [^{125}I]PYY (25 pM) at 25 °C for 120 min. Following three washes, the membrane-bound radioactivity was measured using a TopCount™ microplate scintillation counter (Packard, Meriden, CT). Non-specific binding was determined in the presence of excess cold porcine PYY (1 μM).
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